Type 1 Diabetes and MicroRNA: It's Complicated

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Type 1 diabetes is an autoimmune disease that manifests as impaired insulin secretion, with compounding complications over time. Bhatt et al. (2015) investigate protective mechanisms in survivors of type 1 diabetes by using induced pluripotent stem cells as genetic models, uncovering novel interactions between microRNA and the DNA damage checkpoint pathway.

Immune cell targeting of pancreatic β cells is the putative cause of type 1 diabetes (T1D), thought to be initiated by some genetic predisposition or environmental stress, then exacerbated by a positive feedback loop driven by β cell apoptosis. Antigens released during apoptosis will recruit more immune cells, with the eventual result of total loss of functional β cells (Bluestone et al., 2010). Dysregulation of blood glucose secondary to loss of insulin secretion is the primary physiological consequence of T1D. Blood glucose levels can be managed with insulin replacement therapy, but often this treatment is insufficient to avert cellular complications induced by hyperglycemia. Common sequelae of cellular complications in T1D include heart disease, limb amputation, and blindness.

The unifying hypothesis concerning the biochemical origin of cellular complications of T1D implicates the inhibition of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) function by superoxide accumulation as the causative mechanism (Brownlee, 2001). Resultant shunting of various glycolytic intermediates to alternative pathways in endothelial cells induces apoptosis, leading to the complications associated with microvascular damage such as diabetic neuropathy and nephropathy. Rodent models of T1D, while valuable, incompletely recapitulate some cellular effects of chronic blood glucose dysregulation (Calcutt et al., 2009). Of particular relevance to this study are the difficulties in studying diabetic neuropathy and macrovascular damage in rodent models of T1D, due to fundamental physiological differences between rodents and humans. To skirt these difficulties, Bhatt et al. (2015) employ induced pluripotent stem cell (iPSC) reprogramming from a carefully selected cohort of T1D patients to study the interplay of genetics and susceptibility to the microvascular complications of T1D.

Disease modeling with pluripotent stem cells is a growing field, spurred on by the advent of iPSC reprogramming technology (Cherry and Daley, 2012). iPSCs represent a means to direct testing of a particular patient's genotype in vitro in any number of cell types, without immortalized cell lines or isolation of short-lived primary cells from said patient. In the study at hand, T1D patients who were diagnosed more than fifty years ago were segregated by clinical phenotype in two groups (Figure 1), those with microvascular complications (+C), and those without microvascular complications (−C). The experiment was designed as a discovery effort, to transcriptionally and proteomically profile primary fibroblasts and their cognate iPSCs with the hopes of identifying some factor or pathway differentially expressed in +C patients that predisposed them to accumulating microvascular damage.

The authors found that the miR200 family is upregulated in both the fibroblasts as well as the reprogrammed iPSCs from the +C cohort. The ultimate conclusion from this finding is that many of miR200's targets are transcripts encoding DNA damage checkpoint proteins. The authors demonstrate that there is a concomitant loss of accumulated DNA damage checkpoint proteins in the +C cohort of fibroblasts and iPSCs, as well as an increase in markers of cell death such as caspases and pH2AX.

Rescue of the DNA damage checkpoint pathway in the +C cohort of cells was achieved by knockdown of miR200 via siRNA. miR200 knockdown affected loss of histone H2AX phosphorylation and caspase-3 cleavage, suggesting that the accrual of DNA damage in these primary fibroblasts and reprogrammed iPSCs was entirely a product of miR200-driven suppression of the DNA damage checkpoint pathways. This effect was also sustained throughout neuronal differentiation of the iPSCs. Neurons differentiated from +C iPSCs accumulated pH2AX in the nucleus, whereas −C iPSCs did not. Exogenous overexpression of miR200 in differentiated neurons produced nuclear pH2AX in cell lines derived from all clinical backgrounds. These data strongly associate miR200-mediated downregulation of the DNA damage checkpoint proteins with propensity for developing microvascular complications of T1D.

The findings presented here are provocative for several reasons. For one, miR200 is secreted in exosomes and is detectable in serum, as observed here and in other studies (Le et al., 2014). Le et al. showed that exosome-packaged miR200 derived from metastatic cancer cells could prompt transdifferentiation and metastasis in distal poorly metastatic cancer cells. It is possible that miR200 upregulation in endothelia affected by chronic hyperglycemia could be signaling to other tissues via endocrine action of miR200 in secreted exosomes. If this is indeed the case, then exosome-mediated miRNA signaling could be another mechanism for advancement of the symptoms of microvascular and neuropathological complications of T1D. It was recently demonstrated that miR200 suppression in β cells protects against cell death in a model of type 2 diabetes, supporting the Bhatt et al. (2015) study (Belgardt et al., 2015).

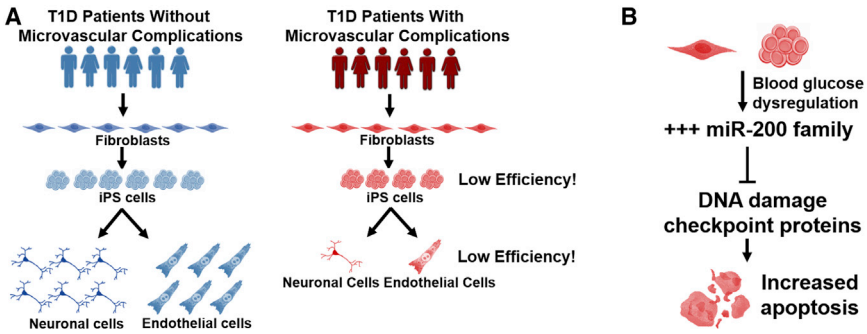

Figure 1. Fibroblasts from T1D Patients with Microvascular Complications Exhibit Decreased iPSC Reprogramming Efficiency Secondary to Upregulated miR200

(A) Bhatt et al. (2015) reprogrammed iPSCs from dermal fibroblasts taken from two groups of patients, both with long-standing (50+ years) T1D. One group suffered microvascular complications (red), while the other group did not (blue). Fibroblasts from the group with diabetic complications exhibited decreased efficiencies both at the reprogramming stage and at the differentiation stage, where the researchers attempted to derive neuronal and endothelial cells from the iPSCs.

(B) Transcriptome analyses revealed that cells obtained from the group of donors suffering from microvascular complications of T1D expressed the miR200 family, concomitant with a decrease in expression of target genes in the DNA damage checkpoint pathways and increased DNA damage accumulation.

The effect of the miR200 family on transdifferentiation of cancer cells (epithelial-mesenchymal transition, EMT) has long been recognized (Korpal et al., 2008), but this could be the first association between miR200 expression and reprogramming and differentiation efficiencies of iPSCs. In the current publication, Bhatt et al. (2015) make note of a disparity in iPSC reprogramming and differentiation efficiency between their two cohorts. Indeed, this disparity is what prompted the transcriptomic studies of the parental fibroblasts, leading to the ultimate finding of miR200 as a factor related to DNA damage repair and apoptosis in fibroblasts and differentiated neurons from +C patients. The miR200 family exerts its control over EMT through the ZEB transcription factors in cancer, and perhaps it is playing some similar role inhibiting reprogramming and differentiation in this study.

Another point of emphasis to discuss in the current study is the persistence of miR200 differential expression in primary fibroblasts, reprogrammed iPSCs, and differentiated neuronal cells. This carryover of expression differentials must be related to some underlying genetic or epigenetic cause, but the authors have yet to discover this mechanism. It is understood that certain epigenetic marks can carry over during the reprogramming process, in a phenomenon referred to as "epigenetic memory" (Vaskova et al., 2013). Careful analysis of whole-genome sequencing and unbiased studies of epigenetic marks could reveal the mechanism behind this robust gene expression signature of the +C cohort.

Bhatt et al. (2015) have uncovered a novel function of the miR200 family in T1D. This may provide a link between endothelial cell metabolism dysfunction during chronic blood glucose dysregulation and downstream effects on other tissues.

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